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Please find below and/or attached an Office communication concerning this application or proceeding.

# Office Action Summary

| Application No. | Applicant(s) |
|---|---|
| 09/819,193 | Carter |
| **Examiner** | **Art Unit** |
| Karen Can Ila | 1642 |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3 months* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1) ☐ Responsive to communication(s) filed on _____.

2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

**Disposition of Claims**

4) ☒ Claim(s) *1-13* _____ is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *1-13* _____ is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved.

12) ☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

**Attachment(s)**

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
18) ☐ Interview Summary (PTO-413) Paper No(s). _____
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other:

## DETAILED ACTION

1. Acknowledgment is made of applications election, without traverse, of Group I, drawn to cellular compositions and methods of culturing cells in vitro.

2. Claims 14-20 are canceled. Claims 1-13 are pending and examined on the merits.

### *Claim Rejections - 35 USC § 101 and 35 USC § 112*

3. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

4. Claims 1-13 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific, substantial asserted utility or a well-established utility.

The disclosed invention is a cellular composition, the CAC-1 cell line, comprising cells isolated from a poorly differentiated uterine cancer which are characterized by a responsiveness to retinoic acid. The specification and prior art (Carter et al, Experimental and Molecular Pathology, 2000, Vol. 69, pp. 175-191) asserts that the CAC-1 cell line will be useful for investigating the effects of retinoids on differentiation induction concomitant with actin reorganization. However, this utility is not a substantial utility as it is an experimental use to attain further information on the CAC-1 cell line itself.

The instant situation is directly analogous to that which was addressed in *Brenner v. Manson*, 148 U.S.P.Q. 689 (1966), in which the court held that:

> The basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility. . . . [u]nless and until a process is refined and developed to this point-where specific benefit exists in currently available form-there is insufficient justification for permitting an applicant to engross what may prove to

> be a broad field. . . . a patent is not a hunting license. . . .[i]t is not a reward for the search, but compensation for its successful conclusion.

The instant claims are drawn to a cell line of as yet undetermined biological significance. The use of a product as the object for further research has been determined by the courts as not supporting patentability. Until some actual and specific use can be attributed to the cell line identified in the specification as CAC-1, one of ordinary skill in the art would be required to perform additional experimentation in order to determine how to use the claimed cellular composition. Thus, there is no immediately apparent or "real world" utility as of the filing date.

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1-13 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific, substantial asserted utility or a well-established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

7. In the event applicant could overcome the rejection under 35 U.S.C. 101 above, claims 7 and 9 would be rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 7 and 9 are drawn to a cellular composition comprising a poorly differentiated uterine tumor having a novel karyotype. The specification fails to provide an enabling disclosure

of the complete evidence that the claimed biological materials are readily available to the public or complete evidence of the deposit of the biological materials.

The specification lacks complete deposit information for the deposit of the CAC-1 cell line. It is not clear that a cell line having the claimed karyotype would be reproducibly isolated without undue experimentation from another individual having endometrial adenocarcinoma or publicly available.

Exact replication of a cell line is an unpredictable event. In this case the isolation of the claimed cell line would be dependent upon having a patient with an endometrial tumor exhibiting the claimed abnormal karyotype. Many cell lines derived from a primary endometrial adenocarcinoma are known in the art, but none possess the claimed karyotype. Although the method for screening tumor cell lines to determine a karyotype is well known in the art, this method will not necessarily lead to the isolation of the claimed cell line if the karyotype of the disclosed tumor does not exist in another patient. It is unclear that one of skill in the art could derive the cell line identical to that claimed. Undue experimentation without reasonable expectation of success would be required to carry out the claimed invention.

Because one of ordinary skill in the art could not be assured of the ability to practice the invention as claimed in the absence of the availability of the claimed CAC-1 cell line, a suitable deposit for patent purposes, evidence of public availability of the claimed CAC-1 cell line or evidence of the reproducibility without undue experimentation of the claimed CAC-1 cell line from another individual, is required.

If the deposit is made under the provisions of the Budapest Treaty, filing of an affidavit or declaration by applicant or assignees or a statement by an attorney of record who has authority and control over the conditions of deposit over his or her signature and registration number stating that the deposit has been accepted by an International Depository Authority under the provisions of the Budapest Treaty, that all restrictions upon public access to the deposit will be irrevocably removed upon the grant of a patent on this application and that the deposit will be

replaced if viable samples cannot be dispensed by the depository is required. This requirement is necessary when deposits are made under the provisions of the Budapest Treaty as the Treaty leaves this specific matter to the discretion of each State.

If the deposit is not made under the provisions of the Budapest Treaty, then in order to certify that the deposits comply with the criteria set forth in 37 CFR 1.801-1.809 regarding availability and permanency of deposits, assurance of compliance is required. Such assurance may be in the form of an affidavit or declaration by applicants or assignees or in the form of a statement by an attorney of record who has the authority and control over the conditions of deposit over his or her signature and registration number averring:

(a) during the pendency of this application, access to the deposit will be afforded to the Commissioner upon request:

(b) all restrictions upon the availability to the public of the deposited biological material will be irrevocably removed upon the granting of a patent on this application:

(c) the deposit will be maintained in a public depository for a period of at least thirty years from the date of deposit or for the enforceable life of the patent of or for a period of five years after the date of the most recent request for the furnishing of a sample of the deposited biological material, whichever is longest; and

(d) the deposit will be replaced if they should become nonviable or non-replicable.

Amendment of the specification to recite the date of deposit and the complete name and address of the depository is required. As an additional means for completing the record, applicant may submit a copy of the contract with the depository for deposit and maintenance of each deposit.

If the deposit is made after the effective filing date of the application for patent in the United States, a verified statement is required from a person in a position to corroborate that the cell line described in the specification as filed is the same as that deposited in the depository,

stating that the deposited material is identical to the biological material described in the specification and was in the applicant's possession at the time the application was filed.

Applicant's attention is directed to In re Lundak, 773 F.2d. 1216, 227 USPQ 90 (CAFC 1985) and 37 CFR 1.801-1.809 for further information concerning deposit practice.

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 10 and 12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 10 recites a relationship between a cell line originating from a specimen and the behavior of the cell line in "substantially equivalent ways" as a cell of "said sample". The sentence has no antecedent basis for "said sample". For purpose of examination, sample will be read as specimen. Further, the specification does not define "substantially equivalent ways", therefore the metes and bonds of the claim cannot be determined.

Claim 12, part d, recites "centrifuging said introductory solution" without providing an antecedent basis for "said introductory solution".

### *Claim Rejections - 35 USC § 102*

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

11. Claims 1, 2, 3 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Carter et al (Anticancer Research, 1997, Vol. 17, pp. 1973-1984).

Claim 1 is drawn to a cellular composition comprising cells isolated from a poorly differentiated uterine cancer. Claims 2 and 3 specifically embody endometrial and adenocarcinoma, respectively. Claim 8 specifies that the cellular composition in grown in vitro as a monolayer.

Carter et al disclose a cellular composition comprising KLE cells which are poorly differentiated endometrial adenocarcinoma cells that grown in a monolayer.

12. Claims 1-6, 8 and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by Gal et al (Gynecologic Oncology, 1982, Vol. 13, pp. 50-57) or Rubin et al (Gynecologic Oncology, 1992, Vol. 45, pp. 273-278) or Grenman et al (Cancer Research, 1988, Vol. 48, pp. 1864-1873).

The embodiments of claims 1-3 and 8 are stated above. Claim 5 embodies a cellular composition of claim 1, wherein said cancer is metastatic. Claim 6 embodies the cell line having a plurality of cells which have at least 46 chromosomes. Claim 10 is drawn to a line of cells originating from s human endometrial adenocarcinoma wherein a plurality of cells behaves in equivalents ways at the morphological, physiological or molecular level as a cell of said specimen.

Gal et al disclose the tumor cell line AC-258, growing as a monolayer and originating from a patient with a poorly differentiated adenocarcinoma of the endometrium. Gald et al disclose that the average number of chromosomes per cell was 64.8. Gal et al disclose that the tumor was a metastatic lesion. Gal et al further disclose that histological evaluation of tumor explants revealed the same moprhological identity as the tumor (Figures 1B-D).

Rubin et al disclose the tumor cell line SK-UT2, growing as a monolayer and established from a poorly differentiated adenocarcinoma hysterectomy specimen. Rubin et al disclose that the majority of cells in the composition had 104-124 chromosomes in addition to four X chromosomes (page 275, under the heading "cytogenetics"). Rubin et al disclose that the patient

subsequently developed metastatic lesions after removal of the primary tumor, the tumor is metastatic. (Page 273, second column, under the heading "Cell line"). Rubin et al further disclose that cultured cells exhibited the same morphological and physiological characteristics as said primary tumor in that the cultured cells, when injected into a nude mouse resulted in a poorly differentiated adenocarcinoma similar to the patients original tumor (page 274, under the heading "morphology").

Grenman et al disclose the tumor cell line UM-EC-1, which grows as a monolayer and is derived from a poorly differentiated adenocarcinoma. Metastatic spread to the lymph nodes was evident at the time the primary tumor was removed. Grenman et al disclose the karyotype of cells from passage two as having from 29 to 82 chromosomes, therefore a plurality of cells had at least 46 chromosomes (page 1867, second column, under the heading "Karyotype of Explant Culture Passage 2". Grenman et al disclose that the cultured cells when injected into a nude mouse form a tumor which exhibits the same marked pleomorphism, abnormal mitotic activity and necrosis which is characteristic of the primary tumor. (Figure 1).

13. Claims 1-4, 6, 8 and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by Sakamoto (J Tokyo Med coll, 1988, Vol. 46, pp. 925-936). The specific embodiments of the claims are listed in section 12, above.

Sakamoto et al disclose the tumor cell line TMCC-1, growing as a monolayer and derived from a poorly differentiated endometrial adenocarcinoma. Sakamoto et al disclose that the modal chromosome number is 65. Sakamoto et al further disclose that the transplantation of the culture TMCC-1 cells into nude mice resulted in a tumor of poorly differentiated adenocarcinoma which resembled the original tumor.

14. Claim 11 is rejected under 35 U.S.C. 102(b) as being anticipated by Carter et al (Anticancer Research, 1997, Vol. 17, pp. 1973-1984).

Claim 11 is drawn to a method of culturing cells in vitro comprising the steps [of] introducing said cells to media comprising a 1:1 mixture of Medium 199 and Ham's F12 supplemented with up to 4% serum, anti-biotics, antimicotics and growth factors; and culturing said cells under conditions for proliferation.

Carter et al disclose a method of culturing human endometrial stromal cell comprising introducing said cells into a 1:1 mixture of Medium 199 and Ham's F12 medium, further comprising penstrep-fungazone, insulin, transferrin, selenium and Mito $^{Tm}$. Albright et al discloses that the mixture was serum free (0% serum) thus meeting the limitation of comprising up to 4% serum.

15. Claims 1-13 are rejected under 35 U.S.C. 102(a) as being anticipated by Carter et al (Experimental and Molecular Pathology, 2000, Vol. 69, pp. 175-191). The embodiments of claims 1-10 have been listed above. Claim 11 is drawn to a method of culturing cells in vitro comprising the steps [of] introducing said cells to media comprising a 1:1 mixture of Medium 199 and Ham's F12 supplemented with up to 4% serum, anti-biotics, antimicotics and growth factors; and culturing said cells under conditions for proliferation. Claim 12 specifically embodies the steps of digesting the cells with collagenase A in the growth medium, said medium comprising Medium 199, Ham's F12, supplemented with fetal bovine serum, bovine calf serum, penicillin, streptomycin, L-glutamine, fungazone, insulin, transferrin and selenium; forming a pellet by centrifugation, introducing the pellet to said growth medium, forming a pellet by centrifugation; plating a supernatant onto a plating dish with said growth medium.

Claim 13 is drawn to a method for culturing a cellular composition comprising cells isolated from a poorly differentiated uterine cancer comprising obtaining a hysterectomy specimen of endometrial adenocarcinoma, placing said specimen in medium 1 including Leibovit L-15 medium, 5% penicillin-streptomycin, 500 ug/ml gentamicin and 2.5 ug/ml fungazone; washing said specimen with HBSS; mincing said specimen into 1 mm pieces; washing said pieces with

HBSS, pelleting said pieces by centrifugation for 3 minutes; exposing said pellet to 2 ug/ml collagenase A in growth medium 2 comprising a 1:1 mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, 5% pen-strep, 4mM glutamine, 2.5 ug/ml fungazone, and 1% insulin-transferrin-selenium solution with vigorous pipetting every 15 minutes; separating a first supernatant and a pellet by centrifugation; plating said first supernatant in growth medium 2; re-exposing said pellet to collagenase A in growth medium 2 for 1 hr at 37 degrees C, pipetting vigorously every 15 minutes, resulting in formation of a second supernatant. Plating said second supernatant in growth medium 2; following approximately one month in culture, replating the cells in growth medium 2; after approximately one week in culture, transferring the cells to at least one plating dish comprising growth medium 2; growing the cells until they become essentially confluent; and, splitting the cells every 3 to 4 days into four fractions.

Carter et al disclose all the specific embodiments claimed for the isolation of the CAC-1 cell line, as well as the phenotype and karyotype of said CAC-1 cells .

***Claim Rejections - 35 USC § 103***

16. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

17. Claims 11-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carter et al (Experimental Cell Research, 1992, Vol. 201, pp. 262-272) in view of Carter et al (Anticancer Research, 1997, Vol. 17, pp. 1973-1984) and Albright et al (Pathobiology, 1997, Vol. 65, pp.

177-183) and Varma et al (In Vitro, 1982, Vol. 18, pp. 911-918) and Freshney et al (The culture of Animal Cells, 1994, pp. 142-146).

Claim 11 is drawn to a method of culturing cells in vitro comprising the steps [of] introducing said cells to media comprising a 1:1 mixture of Medium 199 and Ham's F12 supplemented with up to 4% serum, anti-biotics, antimicotics and growth factors; and culturing said cells under conditions for proliferation. Claim 12 specifically embodies the steps of digesting the cells with collagenase A in the growth medium, said medium comprising Medium 199, Ham's F12, supplemented with fetal bovine serum, bovine calf serum, penicillin, streptomycin, L-glutamine, fungazone, insulin, transferrin and selenium; forming a pellet by centrifugation, introducing the pellet to said growth medium, forming a pellet by centrifugation; plating a supernatant onto a plating dish with said growth medium.

Carter et al (Anticancer Research, 1997) teach a method for culturing poorly differentiated human endometrial adenocarcinoma comprising introducing the cells into a growth media comprising a mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, pen-strep antibiotics, the anti-fungal, amphoterecin B, and the growth factors of L-glutamine, insulin, transferrin and selenium. Carter et al do not teach that the mixture of Medium 199 and Ham's F12 is i a 1:1 ratio.

Albright et al (Pathobiology, 1997, Vol. 65, pp. 177-183) the a method for culturing human endometrial stromal cells obtained from a patient comprising digesting said cells with collagenase, placing said cells in a medium comprising a 1:1 mixture of Medium 199 and Ham's F1 and penstrep-fungazone, insulin, transferrin, selenium and MITO™. Carter et al do not teach the collagenase digestion of the sample in the growth medium or the plating of the supernatant obtained after centrifugation.

Varma et al teach a method of separating human endometrial stromal cells from glands containing epithelial cells comprising digestion of cells with collagenase and centrifugation, wherein the glands containing epithelial cells remain in the supernatant, and the stromal cells are in

the pellet. Varma et al do not teach the culture of said epithelial cells in a medium comprising a 1:1 mixture of Medium 199 and Ham's F1 and penstrep-fungazone, insulin, transferrin, selenium L-glutamine, fetal bovine serum and bovine calf serum. Varma et al do not teach the collagenase digestion of the endometrial sample in the growth medium.

Freshney teaches the digestion of tumor samples by collagenase in growth medium. (Page 145, first column, numbers two and three under "protocol").

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to

1. obtain a tissue sample from a patient having human endometrial adenocarcinoma, digest said tissue sample with collagenase A in growth medium,

2. separate the epithelial cells from the stromal cells by centrifugation, the epithelial cells being present in the supernatant.

3. culture the cells from the supernatant in mixture comprising a 1:1 mixture of Medium 199 and Ham's F1 and penstrep-fungazone, insulin, transferrin, selenium L-glutamine, fetal bovine serum and bovine calf serum.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of

Carter et al (1992) and Varma et al on digestion of human endometrial cells by collagenase and the teachings of Varma et al on the separation of an epithelial fraction from a stromal fraction by centrifugation, wherein the supernatant contains the epithelial fraction;

Fresheny on the digestion of tumor samples by collagenase while in growth medium;

Carter et al (1997) on the growth of a cell line of human endometrial adenocarcinoma cells, KLE, in a medium comprising a 1:1 mixture of Medium 199 and Ham's F1 and penstrep, insulin, transferrin, selenium, L-glutamine, fetal bovine serum and bovine calf serum; and

Carter et al (1992) and Albright et al (1997) on the inclusion of an anti-mycotic in the cell culture medium of human endometrial cells obtained from hysterectomy or autopsy.

18. Claims 11-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carter et al (Experimental Cell Research, 1992, Vol. 201, pp. 262-272) and Carter et al (Anticancer Research, 1997, Vol. 17, pp. 1973-1984) and Albright et al (Pathobiology, 1997, Vol. 65, pp. 177-183) and Varma et al (In Vitro, 1982, Vol. 18, pp. 911-918) and Freshney (the Culture of Animal Cells, 1994) as applied to claims 11-13 above, and further in view of Kischer et al (Cytotechnology, 1989, Vol. 2, pp. 181-186) and Latimer (US 6,074,874) and Kniss et al, (American Journal of Obstetrics and Gynecology, 1997, Vol. 177, pp. 559-567).

Claim 13 is drawn to a method for culturing a cellular composition comprising cells isolated from a poorly differentiated uterine cancer comprising obtaining a hysterectomy specimen of endometrial adenocarcinoma, placing said specimen in medium 1 including Leibovit L-15 medium, 5% penicillin-streptomycin, 500 ug/ml gentamicin and 2.5 ug/ml fungazone; washing said specimen with HBSS; mincing said specimen into 1 mm pieces; washing said pieces with HBSS, pelleting said pieces by centrifugation for 3 minutes; exposing said pellet to 2 ug/ml collagenase A in growth medium 2 comprising a 1:1 mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, 5% pen-strep, 4mM glutamine, 2.5 ug/ml fungazone, and 1% insulin-transferrin-selenium solution with vigorous pipetting every 15 minutes; separating a first supernatant and a pellet by centrifugation; plating said first supernatant in growth medium 2; re-exposing said pellet to collagenase A in growth medium 2 for 1 hr at 37 degrees C, pipetting vigorously every 15 minutes, resulting in formation of a second supernatant. Plating said second supernatant in growth medium 2; following approximately one month in culture, replating the cells in growth medium 2; after approximately one week in culture, transferring the cells to at least one plating dish comprising growth medium 2; growing the cells until they become essentially confluent; and, splitting the cells every 3 to 4 days into four fractions.

For the reasons set forth in section 17 above, Carter et al (1992), Carter et al (1997) Albright et al (1997) and Freshney (1994) teach a method of culturing a poorly differentiated

human endometrial adenocarcinoma cells obtained from a patient comprising digesting tissue specimen obtained from a patient with collagenase in growth medium comprising a 1:1 mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, 5% pen-strep, 4mM glutamine, 2.5 ug/ml fungazone, and 1% insulin-transferrin-selenium solution and culturing the resulting dispersed cells in said growth medium Carter et al (1992) or Carter(1997) or Albright et al (1997) or Fresney (1994) do not teach the placement of the hysterectomy specimen in a medium comprising Leibovits L-15 medium and gentamicin, or the plating of a first and a second supernatant, the growth of the cultures for approximately one month, the replating of the culture in at least one 60 mm dish, and the subsequent 1-4 splitting after confluence and maintenance of the resulting cells by splitting 1:4 every 3 to 4 days.

Varma et al teach a method of separating human endometrial stromal cells from glands containing epithelial cells comprising obtaining a specimen of human endometrium, washing said specimen with HBSS; mincing said specimen into 1 mm pieces; washing said pieces with HBSS, pelleting said pieces by centrifugation; exposing the resulting pellet to .25% collagenase which is effectively the same as 2 ug/ml collagenase A; separating a first supernatant and a pellet by centrifugation; plating said first supernatant containing glands comprising epithelial cells in growth medium; re-exposing said pellet to collagenase A in growth medium 2 for 1 hr at 37 degrees C, pipetting every 15 minutes, obtaining a second supernatant. Plating said second supernatant containing glands comprising epithelial cells (page 912 first column under the heading "Tissue dispersion" to second column , line 26). Thus Varma et al teach the plating of the first and the second supernatant to obtain epithelial cells.

Kischer et al teach the use of Leibovits L15 medium preserves the viability of cells in tissue samples and thus is useful as a transport medium.

Kniss et al teach the culture of human endometrial epithelial cells in medium containing 50 micrograms per ml of gentamicin.

Latimer (US 6,074,874) teaches obtaining a primary culture from a tumor explant. Latimer teaches the maintenance of the primary explant culture in a relatively crowded stated, followed by the disassociation of the cells in the primary culture and the replating of said cells to establish a secondary explant culture. Latimer teaches the splitting of the secondary explant culture to establish cell lines.

Carter et al (1992) teach that the human endometrial stromal cells growing in a medium comprising a 1:1 mixture of Medium 199 and Ham's F12, 1% fetal bovine serum, 3% bovine calf serum, 4mM glutamine, 5% pen-strep and 2 ug/ml insulin were split twice weekly in a 1: 4 ratio.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to transport a hysterectomy specimen of endometrial carcinoma in Leibovits L1-5 medium containing 5% pen-strep, 500 micrograms/ml gentamicin and 2.5 ug/ml fungizone; digest the specimen in growth medium comprising a 1:1 mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, 5% pen-strep, 4mM glutamine, 2.5 ug/ml fungazone, and 1% insulin-transferrin-selenium solution; obtain a first and a second supernatant by subjecting the sample to centrifugation, separating the supernatant from the pellet and redigesting the pellet in said growth medium with collagenase A; plating both supernatants to obtain a plurality of cells; letting the cells grow for about one month; replating the cells onto at least one 60 mm dish, letting the cells reach confluence, and then splitting the cells in a 1:4 ration every 3 to 4 days. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of :

Carter et al (1992), Carter et al (1997) and Albright et al (1997) on the method of culturing a poorly differentiated human endometrial adenocarcinoma cells obtained from a patient comprising digesting tissue specimen obtained from a patient with collagenase in growth medium comprising a 1:1 mixture of Medium 199 and Ham's F12, supplemented with 1% fetal bovine serum, 3% bovine calf serum, 5% pen-strep, 4mM glutamine, 2.5 ug/ml fungazone, and 0.1% insulin-transferrin-selenium solution and culturing the resulting dispersed cells in said growth

medium; and the teachings of Carter et al (1992) on the 1:4 ratio of splitting the human endometrial cells growing in said medium for maintenance of the culture;

Varma et al on the separation of stromal cells from glands containing epithelial cells from a sample of human endometrial tissue;

Kischer et al on the use of Leibovits L15 medium as a transport medium;

Kniss et al on the culture of human endometrial epithelial cells in medium containing gentamicin.

Further, as one of skill in the art would be motivated to protect the specimen from contamination during transport, one of skill in the art would use a higher level of gentamicin in the transport medium than in the medium used during cell proliferation in vitro. During transport, the specimen is exposed to the transport medium for a short time, and not all of the cells in a specimen will be in contact with the transport medium as contrasted with tissue cells growing in a monolayer, where all cell are exposed to the medium, therefore, the toxic effects induced by high levels of gentamicin will be minimal when used in the transport medium, but the specimen will be effectively protected from transport-induced contamination.

***Conclusion***

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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